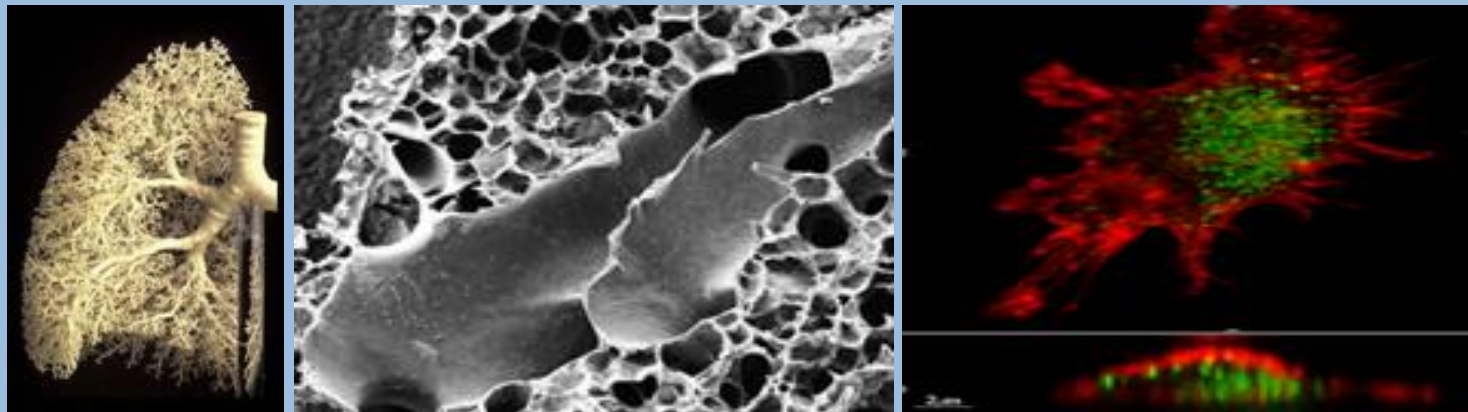


4th International Workshop
Nanoparticle Emissions from Heavy-Duty Vehicles
In Memoriam Professor Yoram Zvirin
Faculty of Mechanical Engineering, Technion, Haifa
June 21, 2016

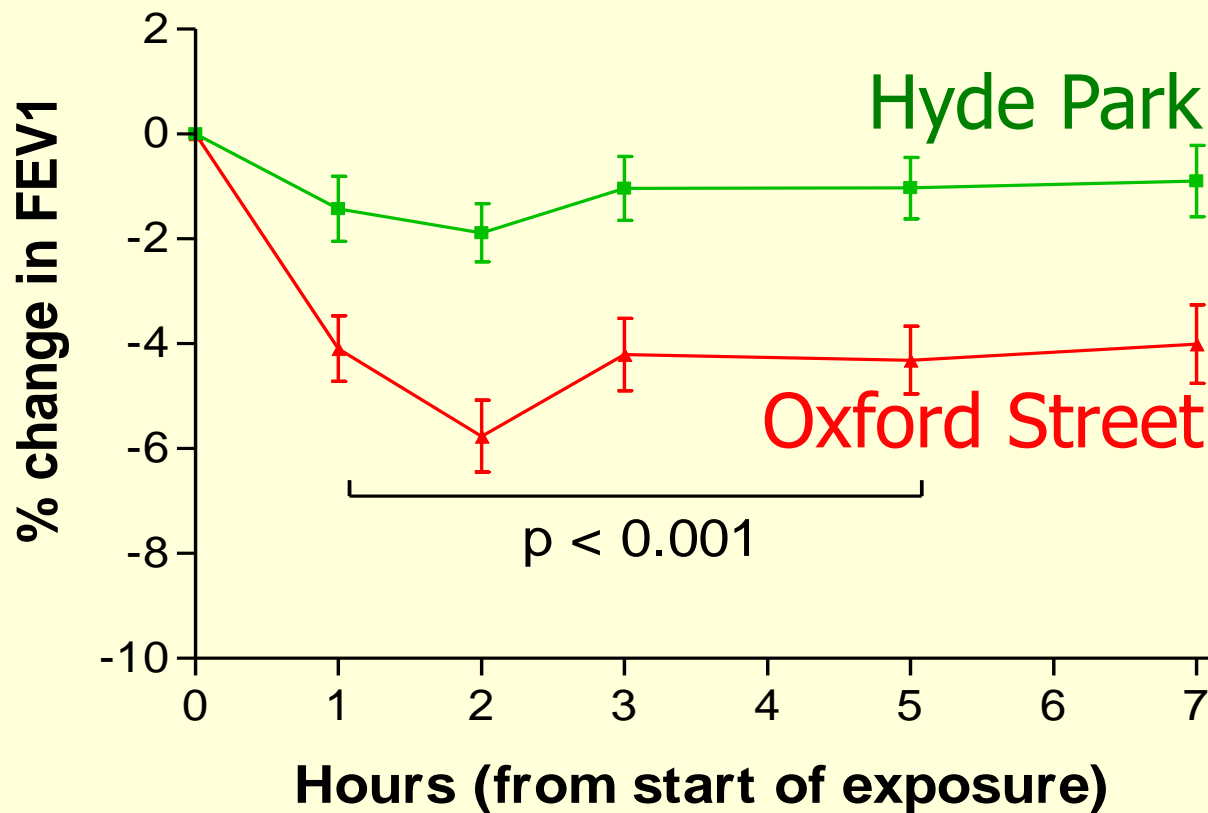
HEALTH EFFECTS OF COMBUSTION GENERATED ULTRAFINE PARTICLES (UFP)

HOW UFP CAN ENTER THE HUMAN ORGANISM – SIZE MATTERS

Peter Gehr, PhD
Prof. em.
University of Bern
Bern
Switzerland



LUNG FUNCTION OF ASTHMATICS WHILE WALKING ALONG THE DIESEL BUS ROUTE OXFORD STREET, THROUGH HYDE PARK



WHO (IARC):

- Diesel exhaust is carcinogenic, June 12, 2012
- Air pollution is carcinogenic, October 17, 2013

Courtesy:

Nino Künzli

Swiss Tropical and
Public Health Institute
Basel, Switzerland

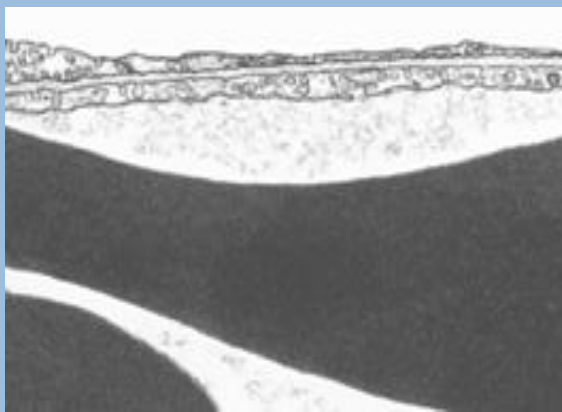
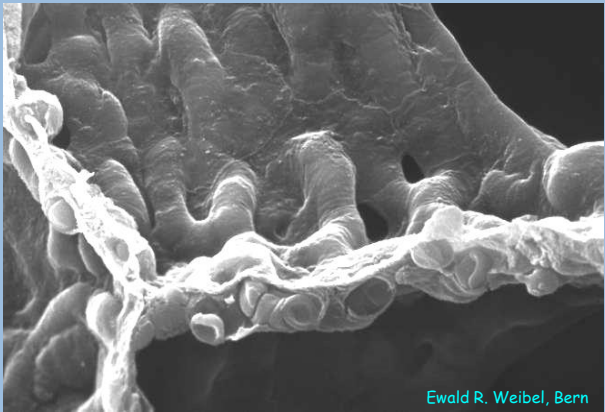
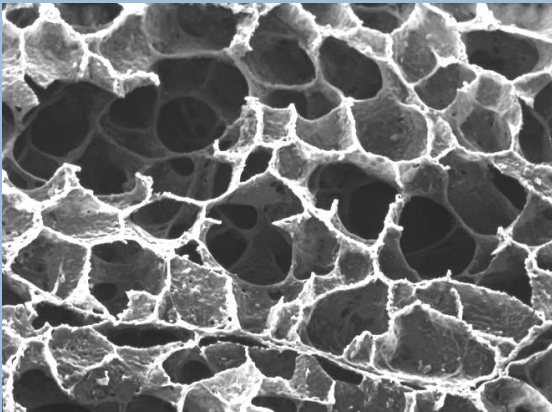
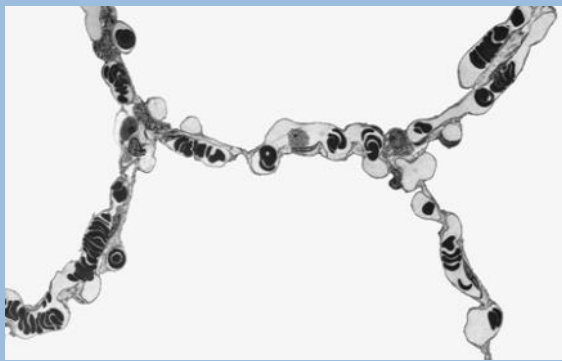
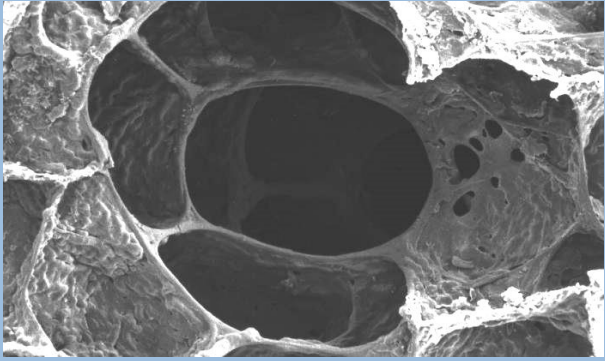
McCreanor et al, NEJM 2007

THE LUNG: MAIN PORTAL OF ENTRY FOR NANOPARTICLES

HUMAN LUNG



Gehr et al., Respir. Physiol., 1978



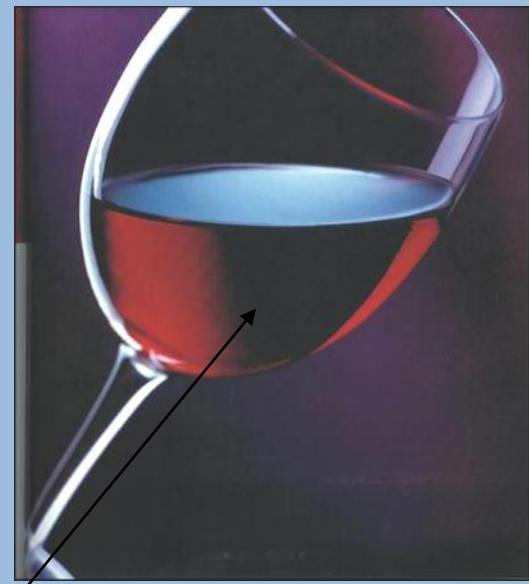
Ewald R. Weibel, Bern

DID YOU KNOW THIS ABOUT THE HUMAN LUNG?



Tennis field

450 Mill. alveoli (M. Ochs, Univ. of Bern)
with a surface area of **140 m²**
(diameter ¼ mm, gas-exchange region 80-90%)



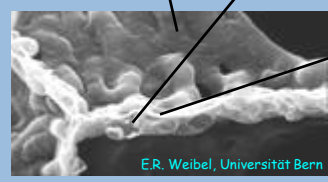
Red wine glass

Volume of capillary blood involved in gas exchange: **210cm³**



1/100 of the thickness of a hair

Thickness of tissue barrier: **<1µm**

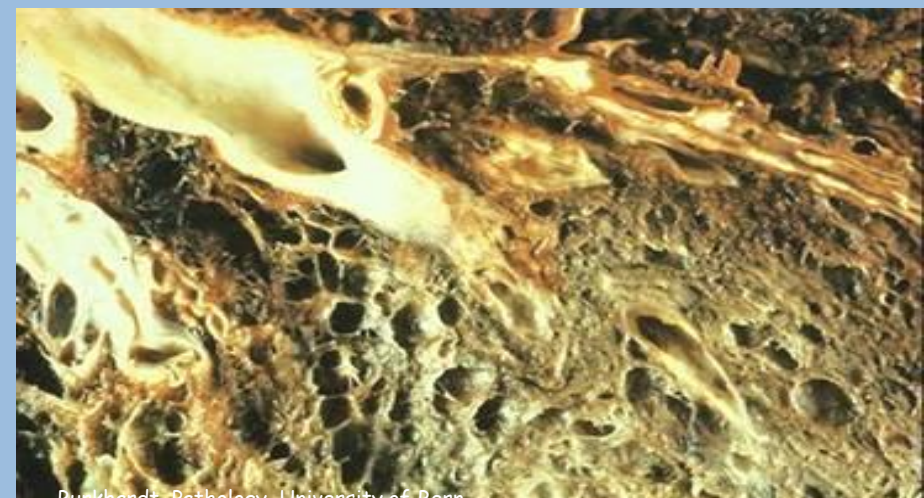


E.R. Weibel, Universität Bern

(B. Rothen-Rutishauser, Universität Bern)

Gehr et al., Respir. Physiol., 1978

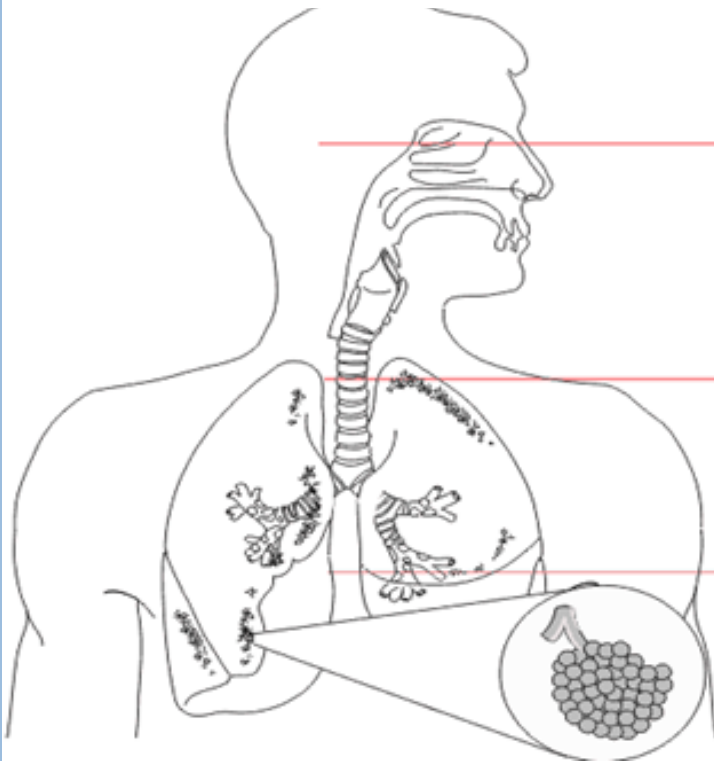
HEALTHY/«CLEAN» LUNG AND POLLUTED LUNG



PARTICLE DEPOSITION IN THE LUNG

PENETRATION OF PARTICLES INTO THE LUNG

Preferential deposition of particles and nanoparticles



Location	Particle size
Upper Airways	5-10 μm
Trachea	3-5 μm
Bronchi	2-3 μm
Bronchioles	1-2 μm
Alveoli	<1 μm (incl. nanoparticles)

⇒ The smaller the particles the deeper they penetrate into the lung:

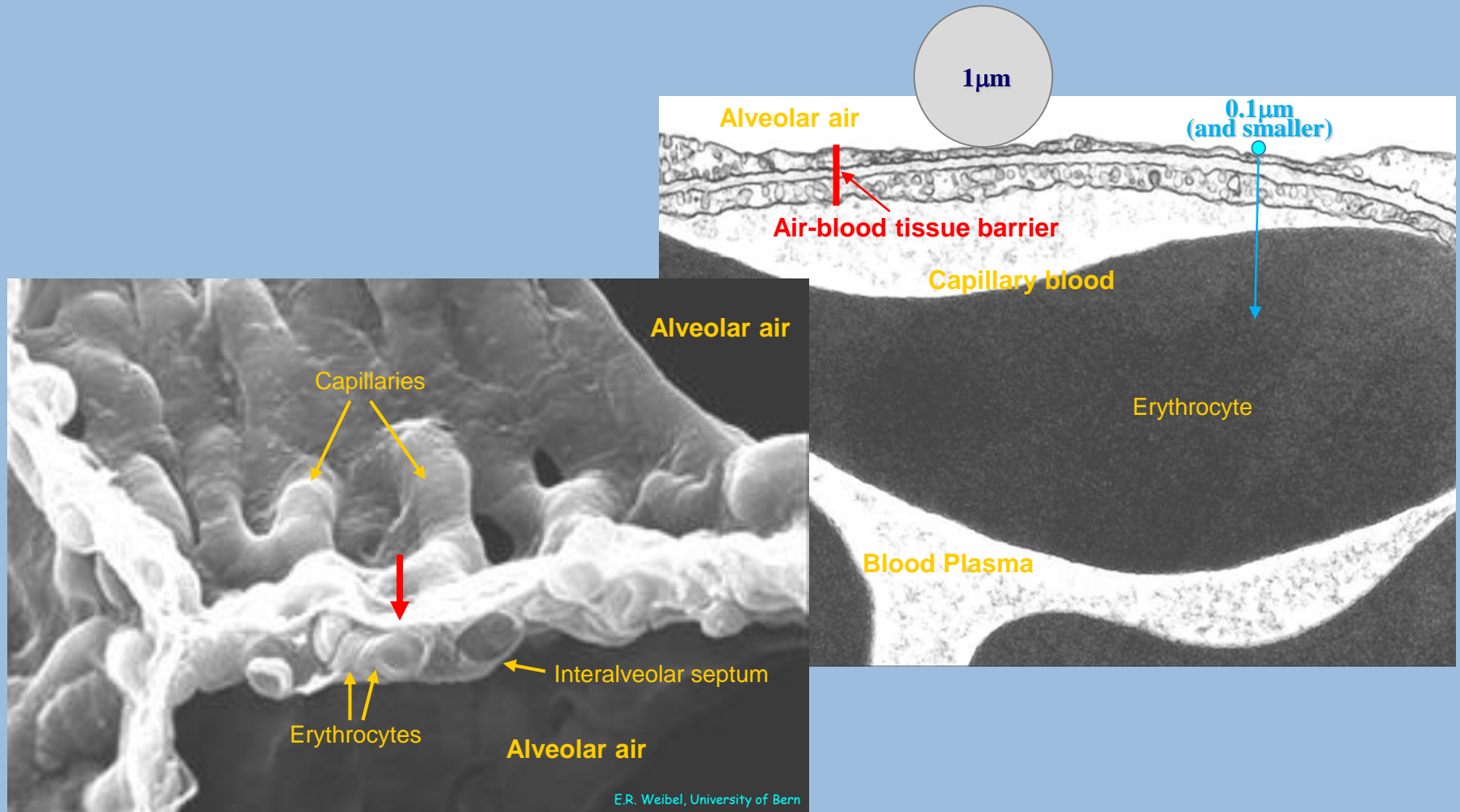
(1) There, **nanoparticles** may penetrate into tissue and cells (organelles, nucleus).

(2) There, **nanoparticles** may translocate into the cappillary blood.

(3) By the blood circulation, **nanoparticles** are transported to other organs.

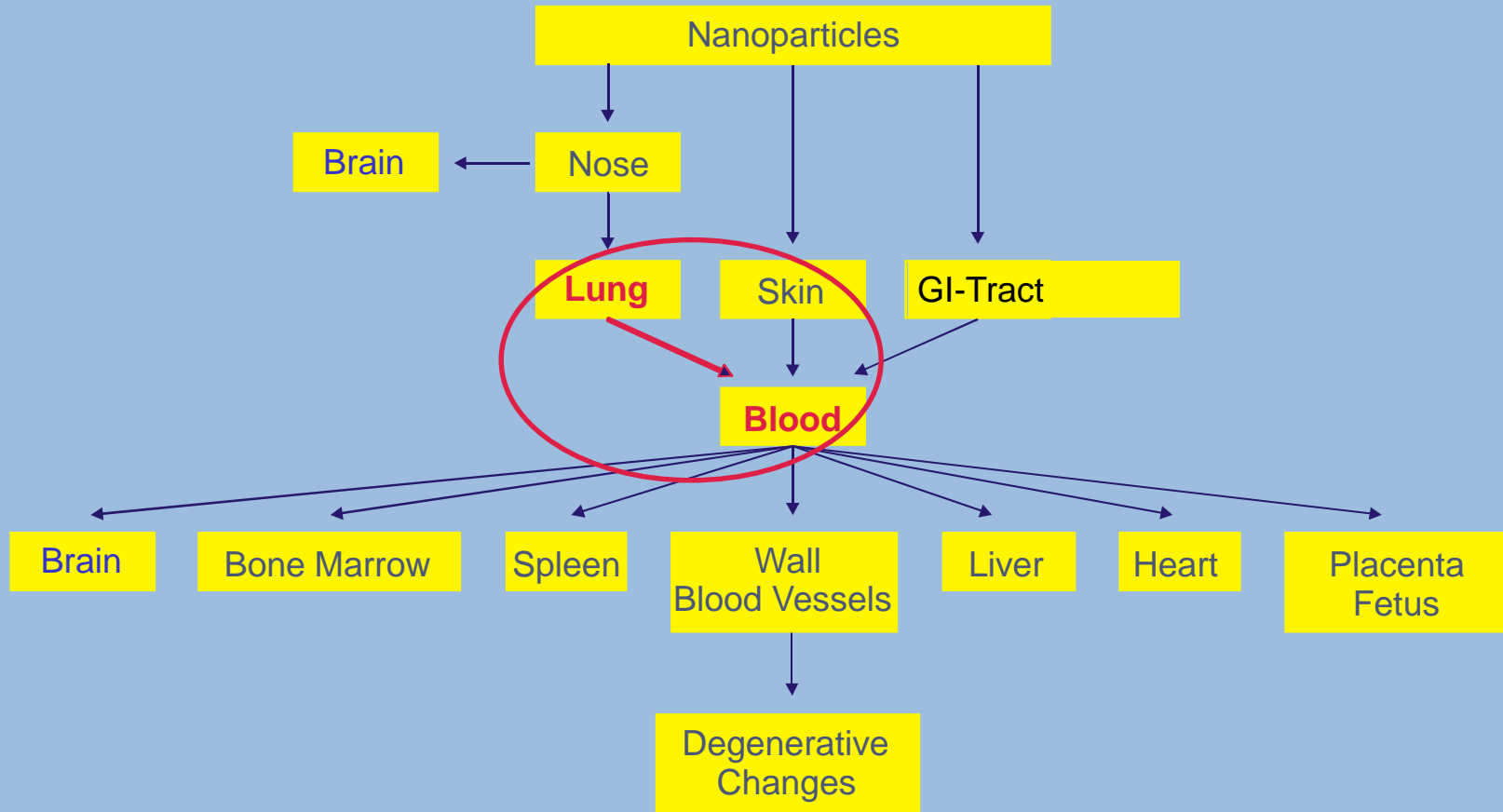
TRANSLOCATION OF NANO-PARTICLES FROM AIR INTO BLOOD

(THROUGH AIR-BLOOD TISSUE BARRIER)



TRANSLOCATION I

WITH BLOOD CIRCULATION TO OTHER ORGANS

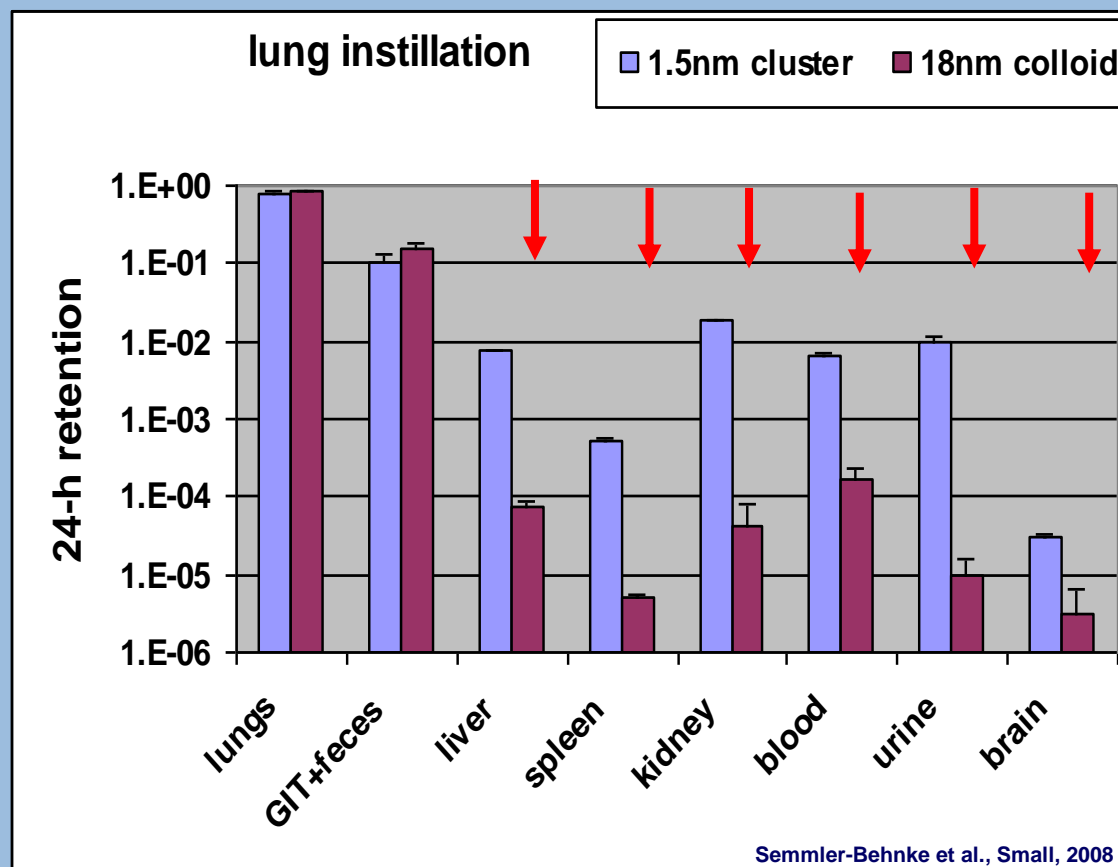


TRANSLOCATION II

AMOUNT OF NANOPARTICLES IN OTHER ORGANS


Intratracheal instillation in WKY rats
1-10 µg ¹⁹⁸Au particles in 50 µL saline, negative ionic surface charge
of particles: 1 · 10¹⁴ (1.4 nm cluster) 2 · 10¹¹ (18 nm colloid)
G. Schmid, Univ. of Essen, Germany

➤ Mass fractions of gold nanoparticles in different organs **after 24 h**



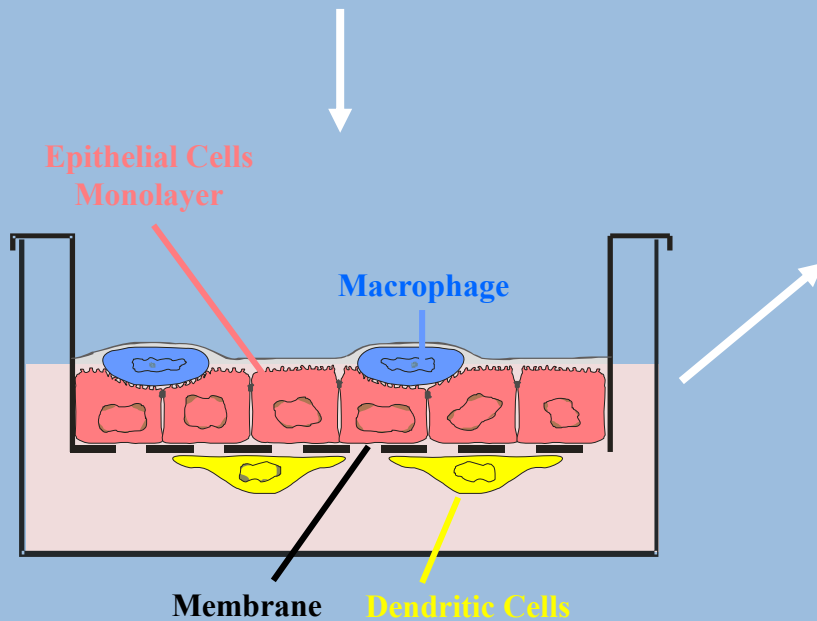
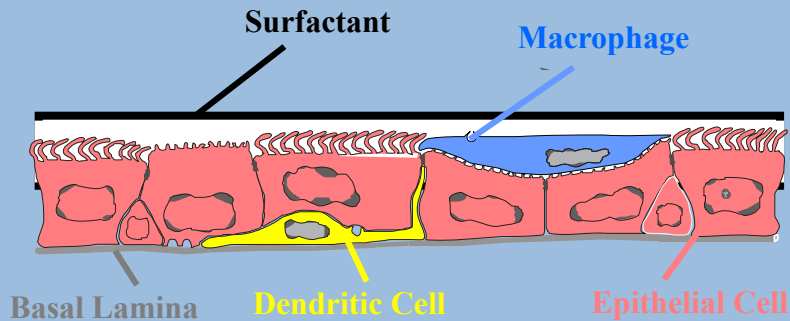
Courtesy:
W.G. Kreyling
(Helmholtz
Center Munich)

Focus Network
Nanoparticles and Health

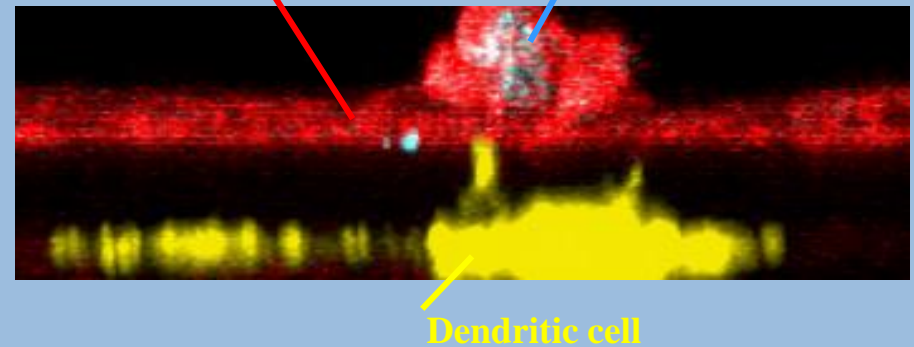
CPC  iLBD
Institute of
Lung Biology and Disease

HelmholtzZentrum münchen

THE TRIPLE CELL CO-CULTURE MODEL TO TEST UFP – CELL INTERACTION

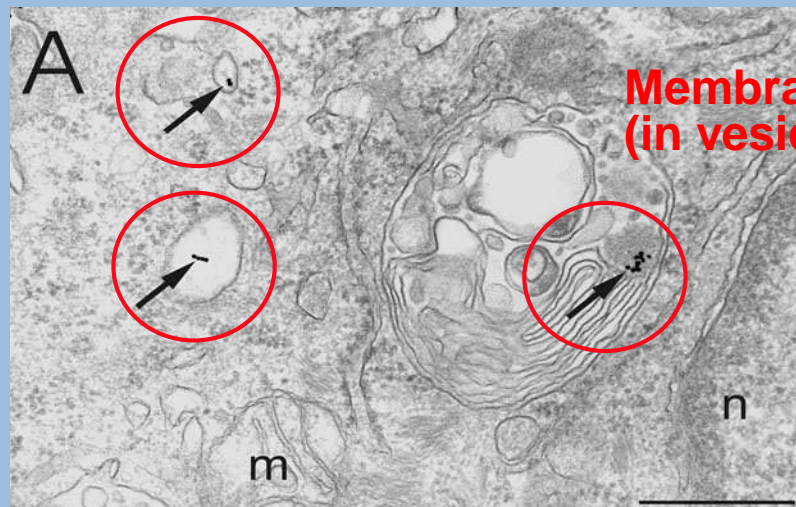


Epithelial cell monolayer
(A549 or 16HBE cells) **Macrophage**



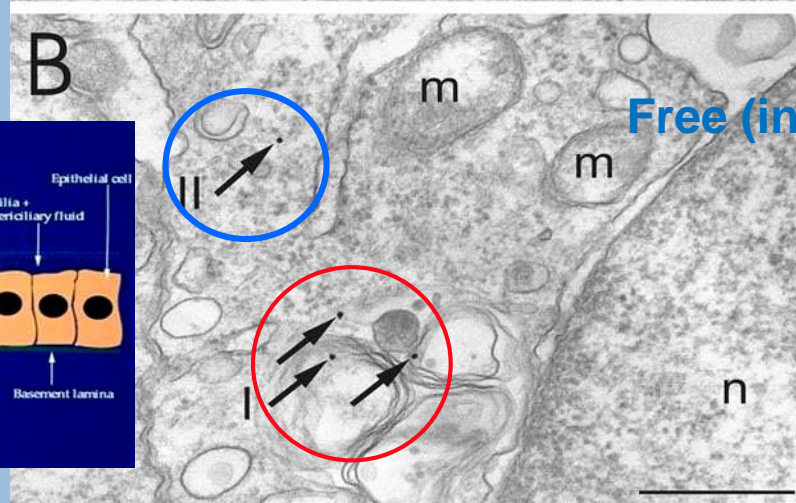
Rothen-Rutishauser et al., *Am. J. Respir Cell Mol. Biol.* 32: 281-899, 2005
Rothen-Rutishauser et al., *Expert. Opin. Drug Metab. Toxicol.* 4: 1075-1089, 2008

NANOPARTICLES (UFP) IN CELLS



Membrane bound
(in vesicles/lysosomes)

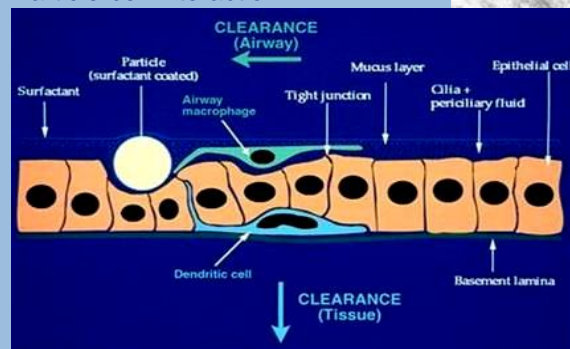
Plain Au nanoparticles



Free (in cytosol)

PEG coated Au nanoparticles
→ more nanoparticles in
cytosol

Particle-cell interaction



Brandenberger et al., Small 2010

WHAT SHOULD BE CONSIDERED

- Diesel exhaust and air pollution were declared **carcinogenic** (UFP)
- Distance to source of air pollution (e.g. traffic) is crucial (1st slide)
- **Filters** contribute substantially to reducing adverse health effects from diesel exhaust particles (>99% particles incl. UFP removed from exhaust)
- **Effects of UFP:**
 - **UFP may enter cells and tissue very easily**
 - **UFP may enter the blood circulation in the lungs and translocate to secondary organs -> the lung is the main portal of entry for UFP**
- **Effects on lungs:**
 - **Reduced pulmonary function in adults (asthmatics) (1st slide)**
 - **Reduced development and function of lungs in neonates (not shown)**
- ***Speculations: Translocation through internal tissue barriers e.g. Blood-brain-barrier (Alzheimer's disease?) a.o.***

TRAFFIC RELATED PM/UFP FROM HIGHWAY 405 (LA) CAUSE ATHEROSCLEROSIS IN MICE

Araujo et al, Circul Res 2008

Exposure:

40 days

5h / day

3 days / week

Toxicology example

Picture from

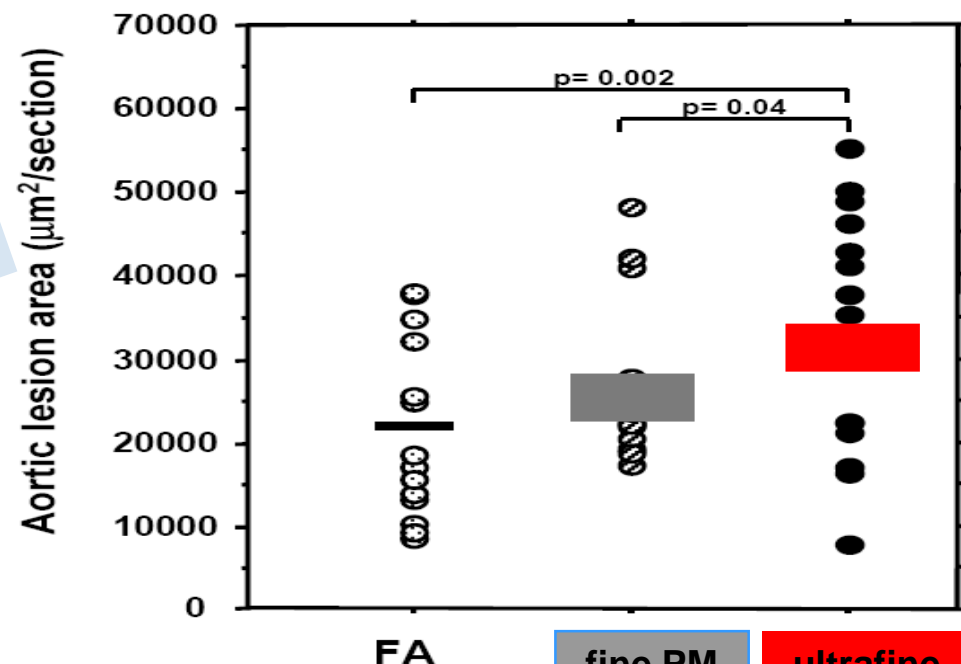
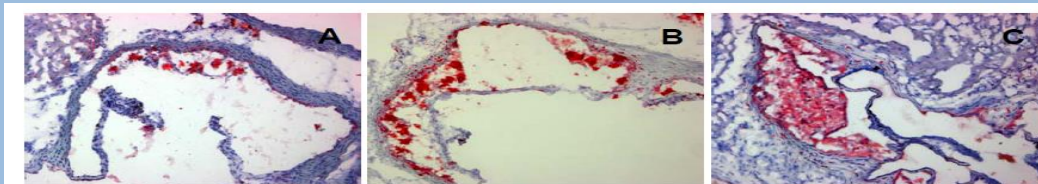
Nino Künzli, MD, PhD; MPH

Professor and Deputy Director

Swiss Tropical and Public Health Institute, Basel

Dean, Swiss School of Public Health, Zurich

Switzerland



fine PM
(PM_{2.5})

ultrafine
PM
(PM_{0.1})

HEALTH EFFECTS OF UFP?

- Ischemic **Heart Disease** mortality in the Californian Teacher's Study
Ostro et al; Env H Perspect 2015
- **Risk of mortality** in association to long-term exposure to traffic-related air pollution. European Studies
- Elemental carbon or Black Smoke are associated with **all-cause mortality** (8 cohort studies)
Review by Hoek et al , Env Health 2013
- **Life expectancy** of reduction in PM2.5 mass ...
- **Lung growth** was affected by home outdoor levels of **PM2.5**
(Southern Californian Children's Health Study, Gauderman et al, NEJM 2004)

A NEW long-term effect study with UFP available!
But a pollutant model with UFP, controlling for PM2.5

Are «effects of NO2» due to ultrafine particles...?
... are the «effects of EC» explained by (unmeasured) UFP?

... are the «effects of EC» explained by (unmeasured) UFP?
... but same associations with elemental carbon... and ... no UFP data available.... – but might look similar... ?!

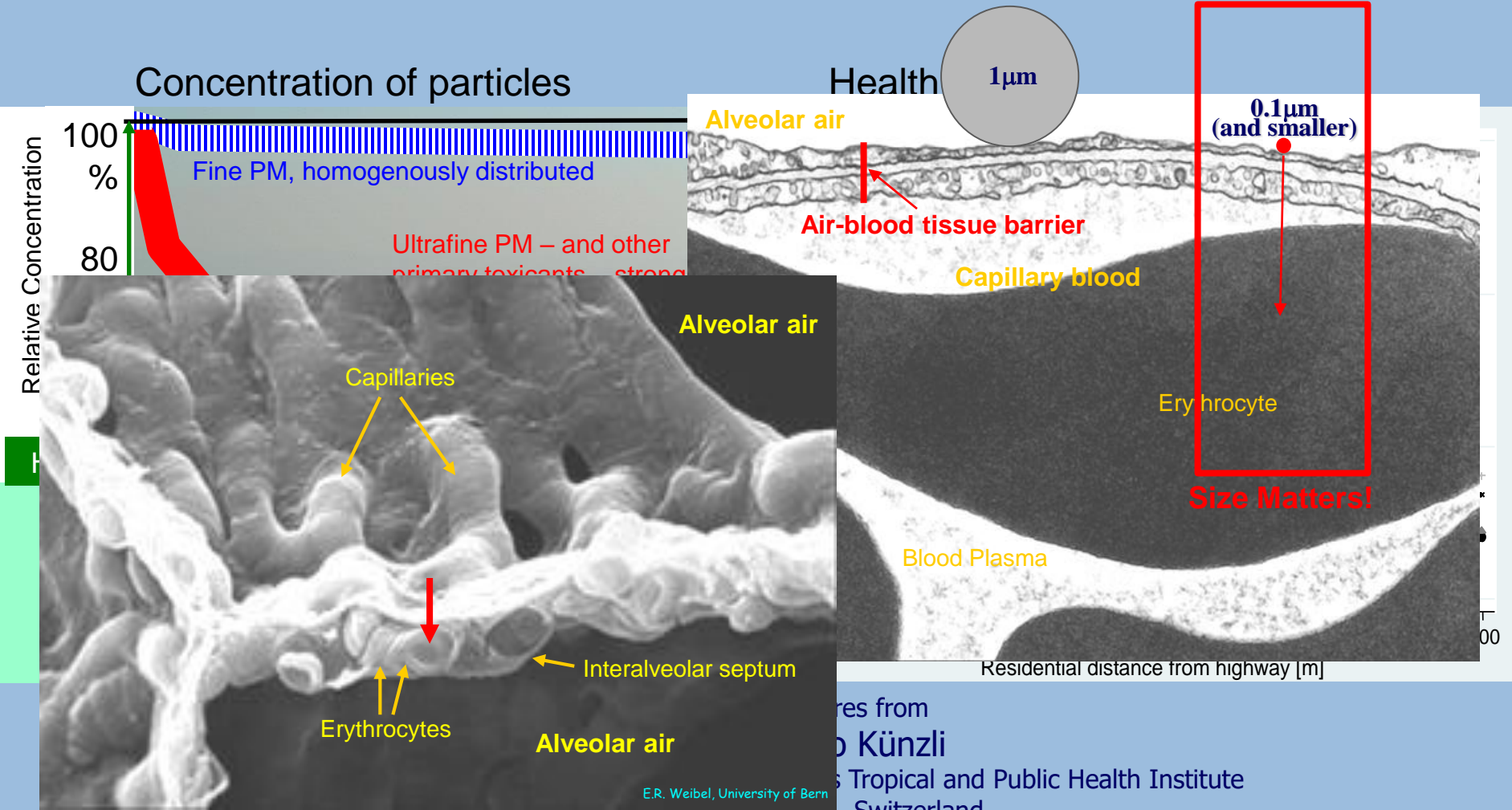
Courtesy
Nino Künzli, MD, PhD; MPH
Professor and Deputy Director
Swiss Tropical and Public Health Institute, Basel, Switzerland

WHAT IS THE BASIS FOR HEALTH EFFECTS?

- **risk = f(hazard, exposure_{time})** for a given size
- **effect = f(dose, time_{exposure/after exposure})** for a given size
- **Interaction of nanoparticles (UFP) with biological systems is primarily a function of size: *size matters!***
peneatration, translocation, effect/reaction
- **Important are furthermore:** material, corona, agglomeration etc.

CONCENTRATION OF PARTICLES AND HEALTH - DISTANCE FROM BUSY ROAD

SIZE MATTERS!



res from
Künzli
Tropical and Public Health Institute
Basel, Switzerland

ACKNOWLEDGEMENTS



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Today: RMS Foundation R. Mathys

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Today: Fed. Dpt. Home Affairs

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Today: Fed. Office of Public Health

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Sandra Frank

Andrea Stokes

Barbara Tschirren

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University of Calgary, Canada

Samuel Schürch

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EMPA: St.Gallen

IST: Lausanne

Helmholtz Zentrum: München

Universität Ulm: Ulm

Universität Marburg: Marburg

Heriot-Watt University: Edinburgh

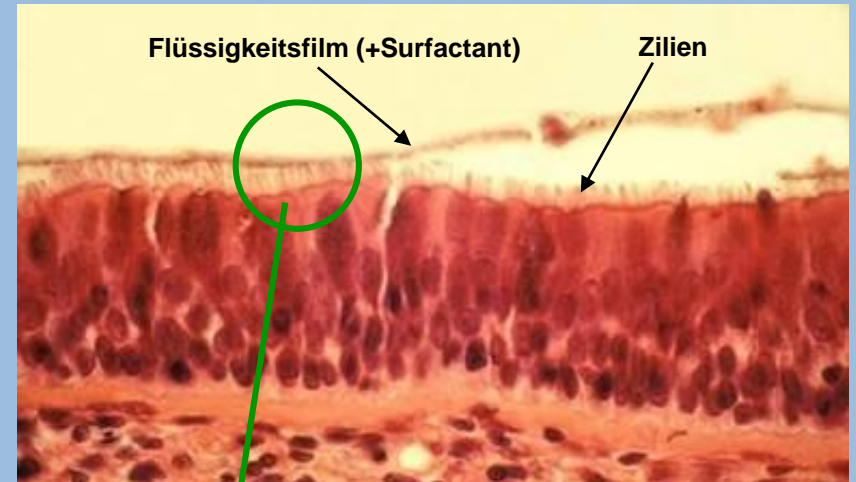
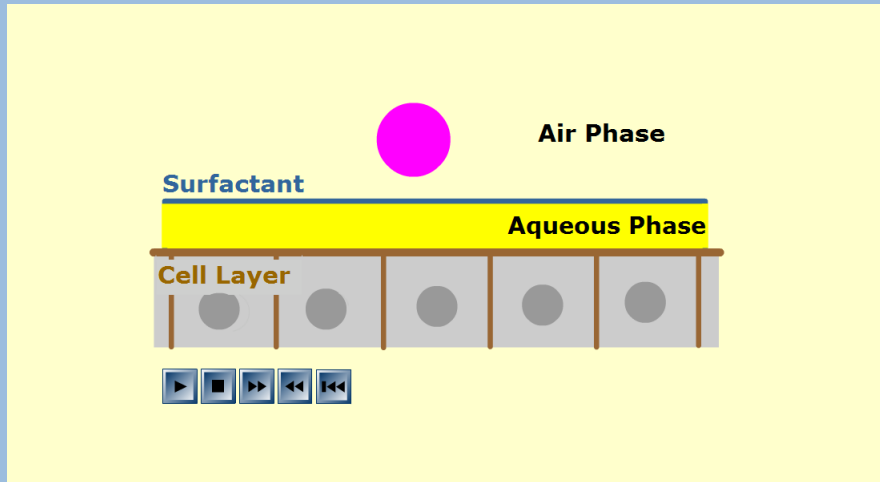
Nino Künzli, MD, PhD; MPH

Professor and Deputy Director
Swiss Tropical and Public Health
Institute, Basel, Switzerland

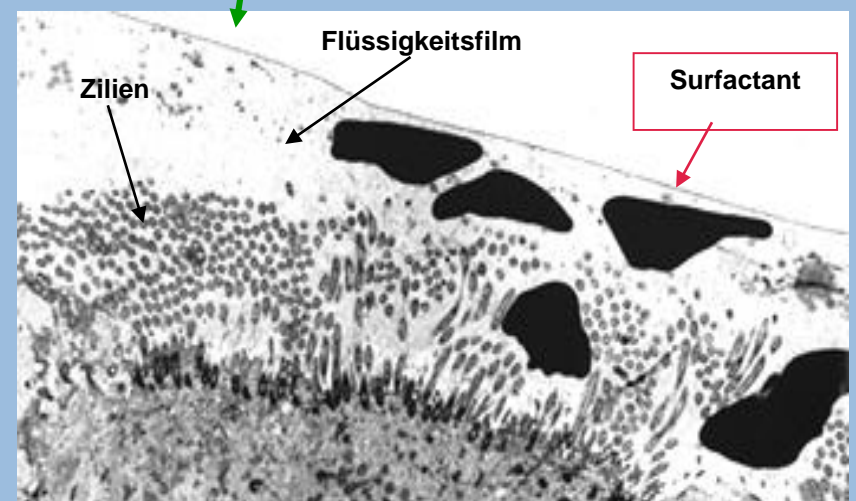
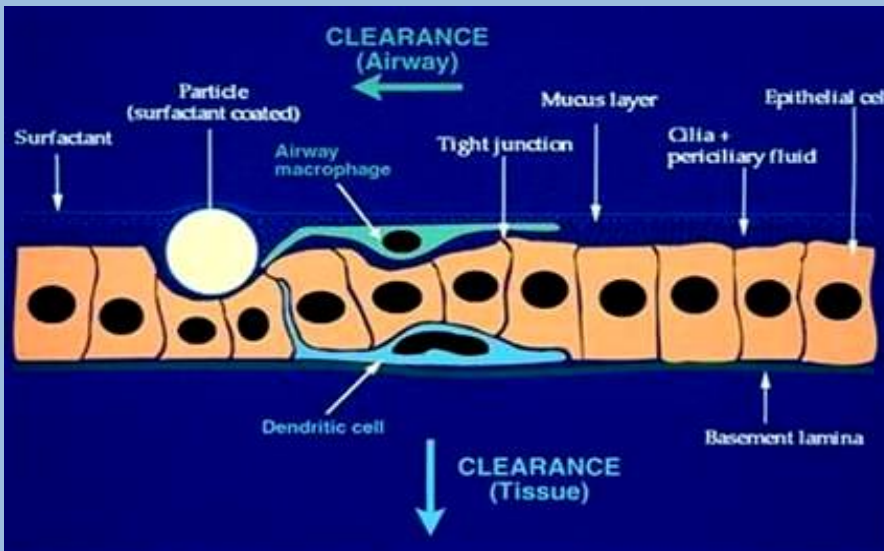
Sponsoring



PARTICLE DISPLACEMENT (SURFACTANT)



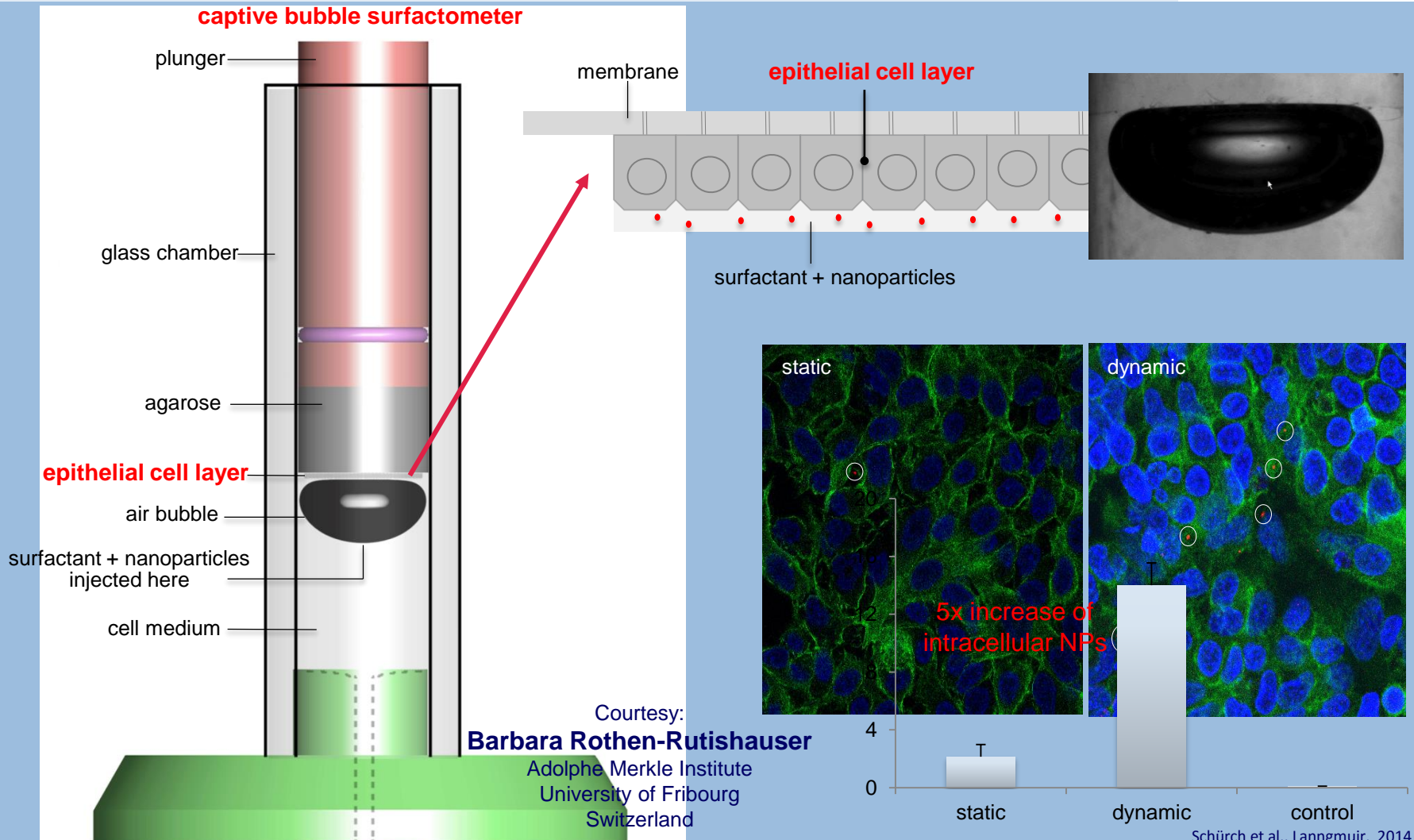
S. Schürch,
S. Tschanz,
Univ. Bern



Gehr et al., J. Aerosol Med., 1990
Schürch et al., Respir. Physiol., 1990
Gehr et al., J. Aerosol Med., 1996

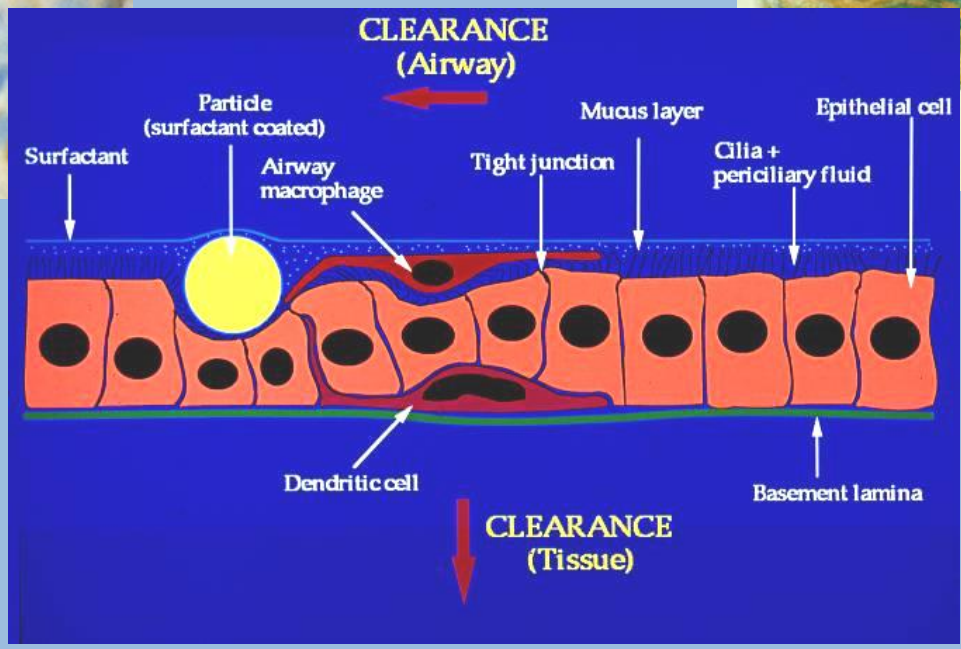
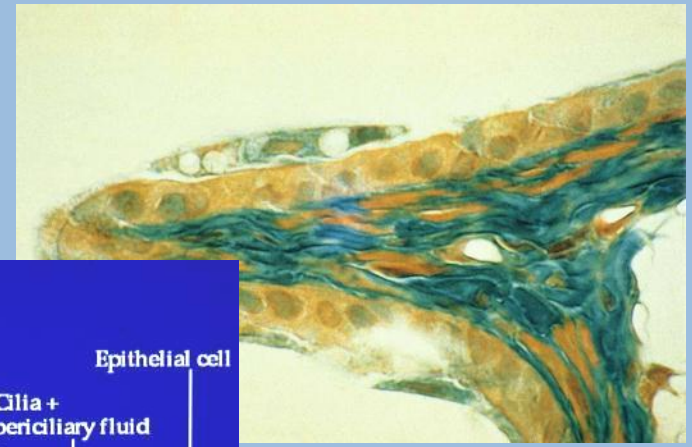
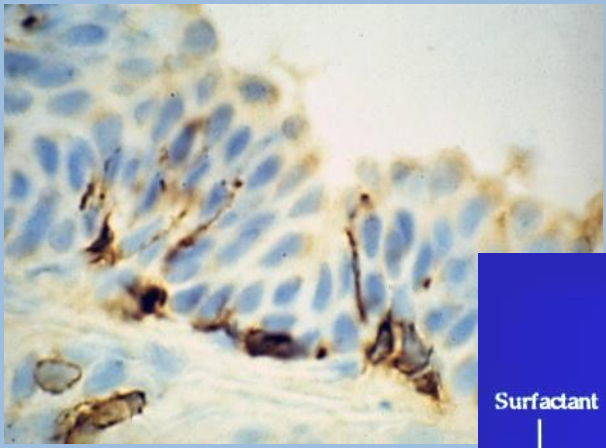
IN VITRO MODEL: BREATHING MOVEMENTS MAY STIMULATE NANOPARTICLE UPTAKE BY CELLS

Courtesy David Schürch, Adolphe Merkle Institute, University of Fribourg

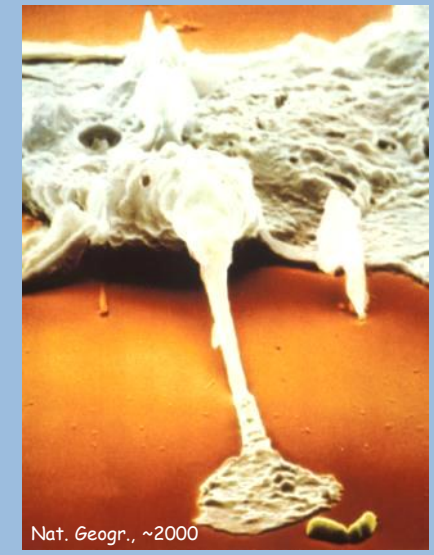


MAIN ACTORS ARE CELLS

EPITHELIAL CELLS, MACROPHAGES, DENDRITIC CELLS ...

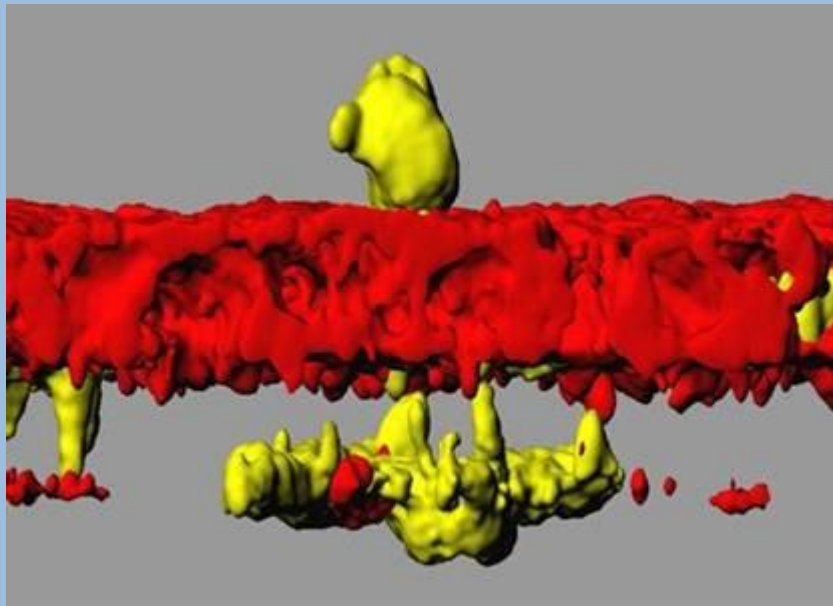


Nat. Geogr., ~2000

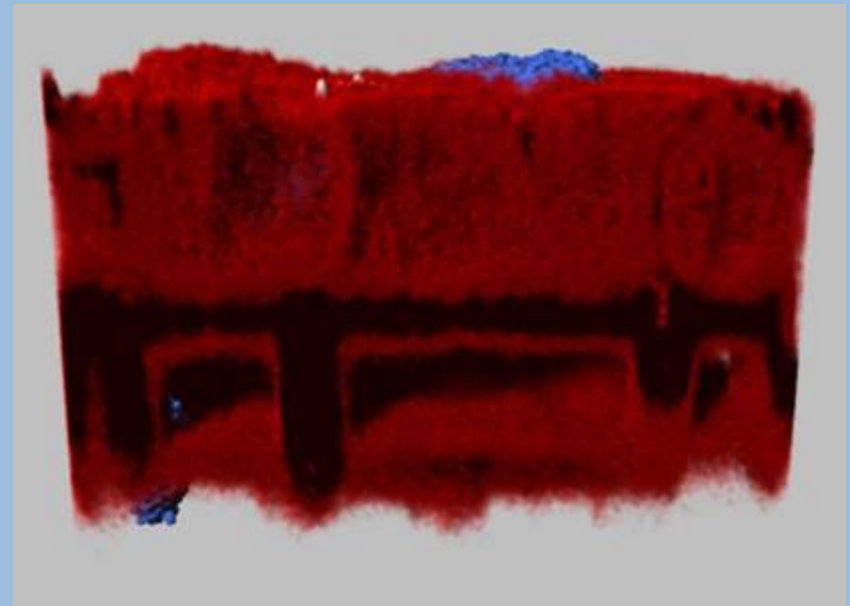


Nat. Geogr., ~2000

STRUCTURAL VICINITY OF DENDRITIC CELLS AND MAKROPHAGES (THROUGH THE EPITHELIAL CELL LAYER)



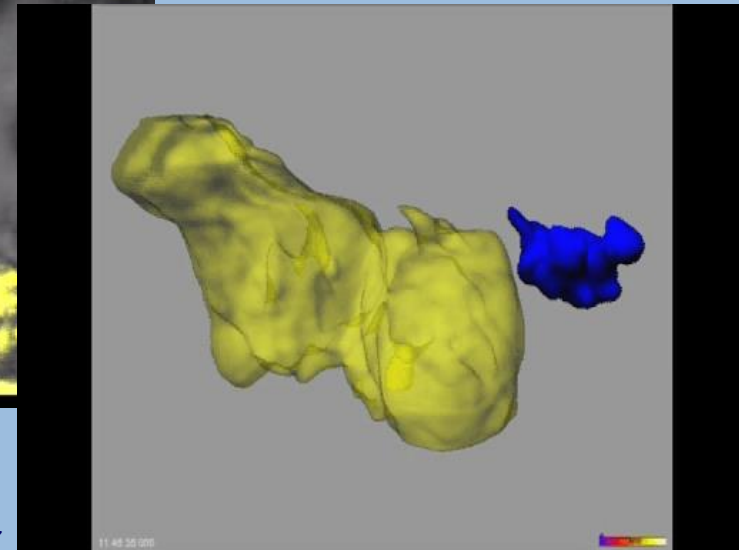
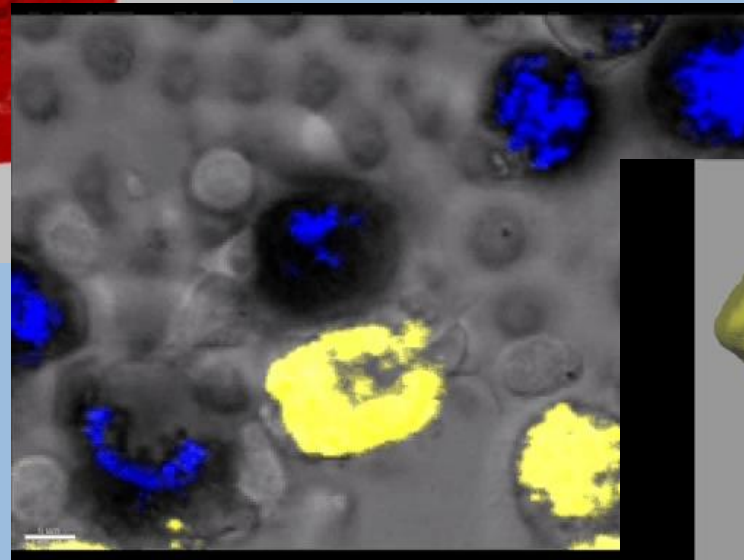
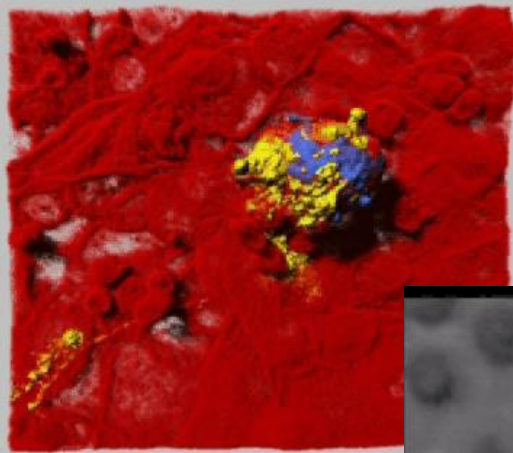
Blank et al., AJRCMB 36: 669-677, 2007



Deconvolution technique
IMARIS 3D&4D Image Analysis Software
Bitplane AG, Scientific Software

CELL-CELL INTERACTIONS

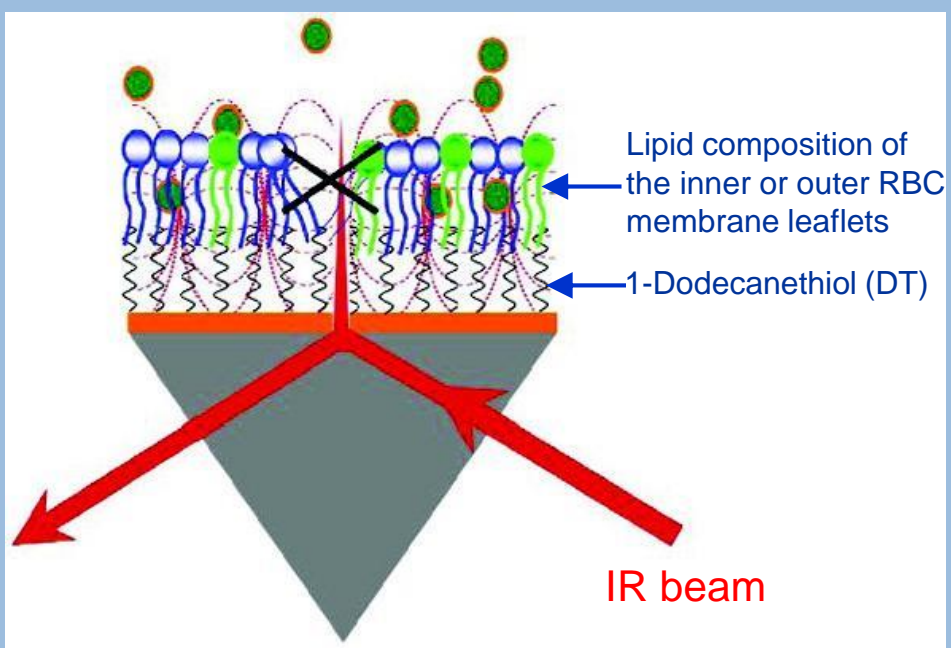
CELLULAR INTERPLAY ->THE CELLS DO
COLLABORATE!



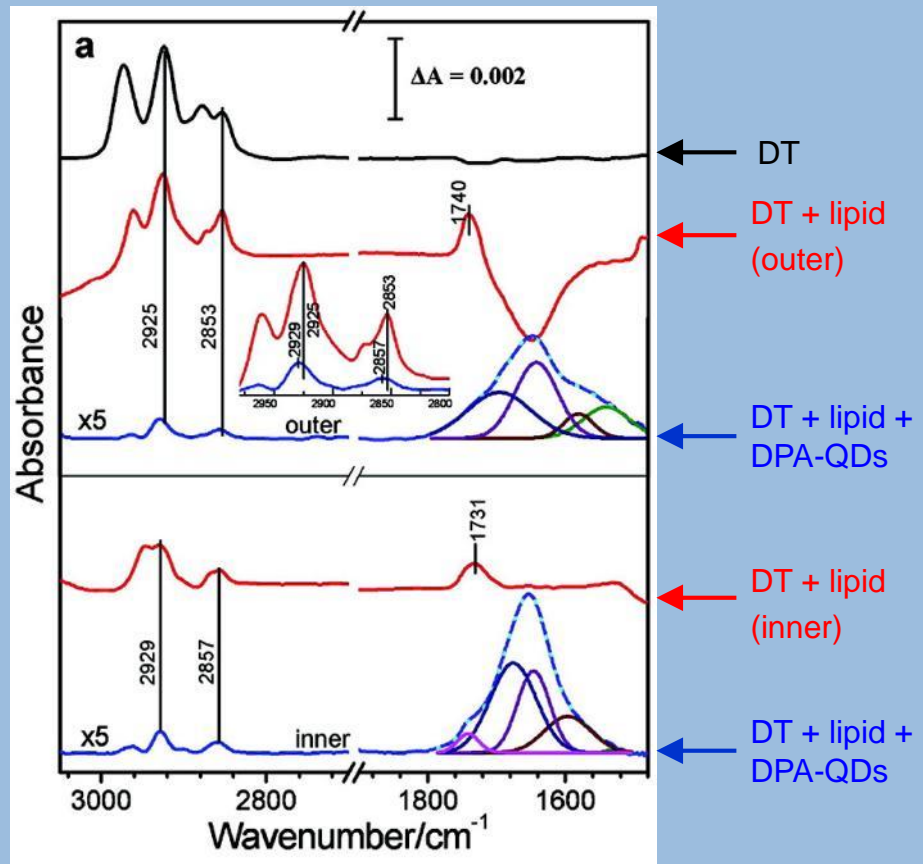
Blank et al., Am. J. Respir. Cell Molec. Biol., 2007

... AND AN ANSWER:

ELECTROCHEMISTRY AND SURFACE-ENHANCED INFRARED ABSORPTION SPECTROSCOPY ON MODEL MEMBRANES (DAP-QDs)



Electrochemistry: voltammograms indicate that lipid layers do not conduct current upon DPA-QD exposure → no holes formed!



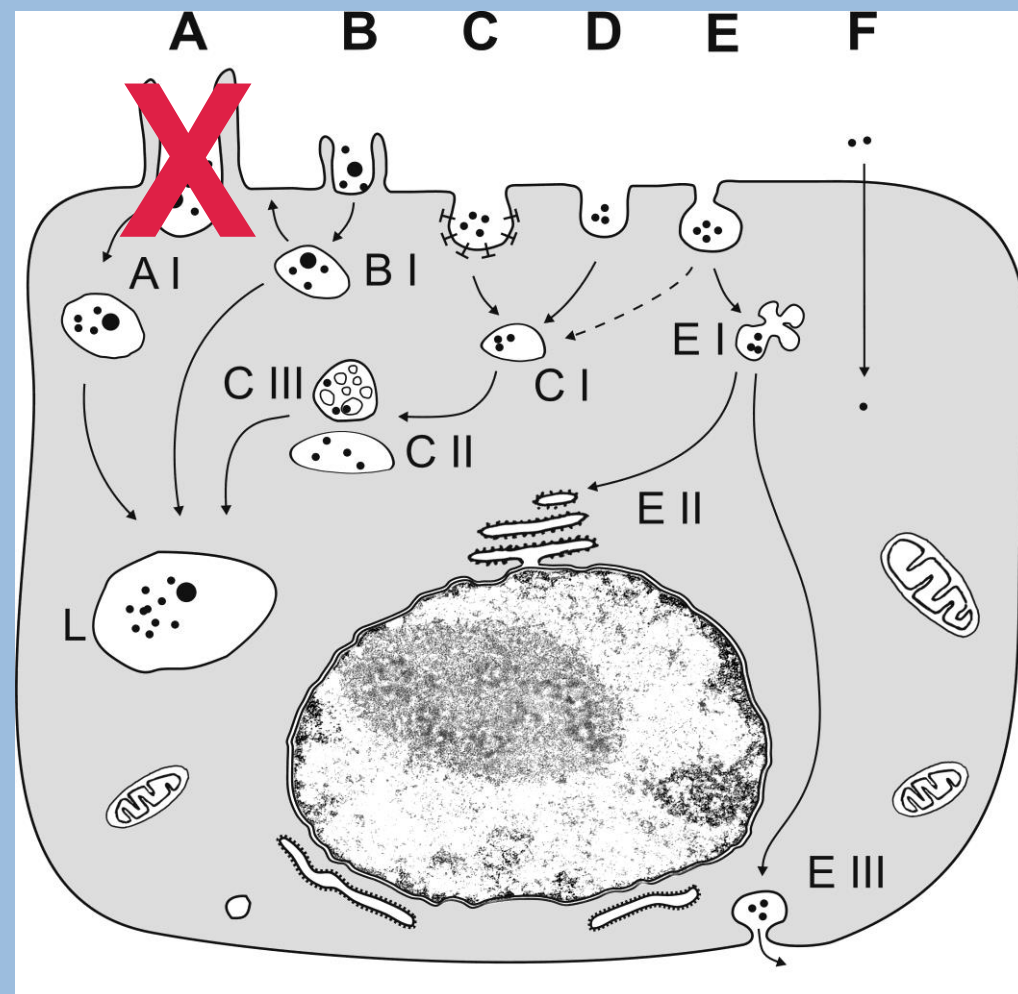
SEIRAS: Membrane flexibility is enhanced in the presence of DPA-QDs

Courtesy:
G.U. Nienhaus,
Institute of Applied Physics, KIT

Wang et al., *ACS Nano* 6 (2012) 1251-1259

(Rothen-Rutishauser et al., *Environ. Sci. Technol.*, 2006)
(Rothen-Rutishauser et al., In Donaldson and Borm, Taylor&Francis, 2007)

A BURNING QUESTION: HOW DO NANOPARTICLES ENTER CELLS?

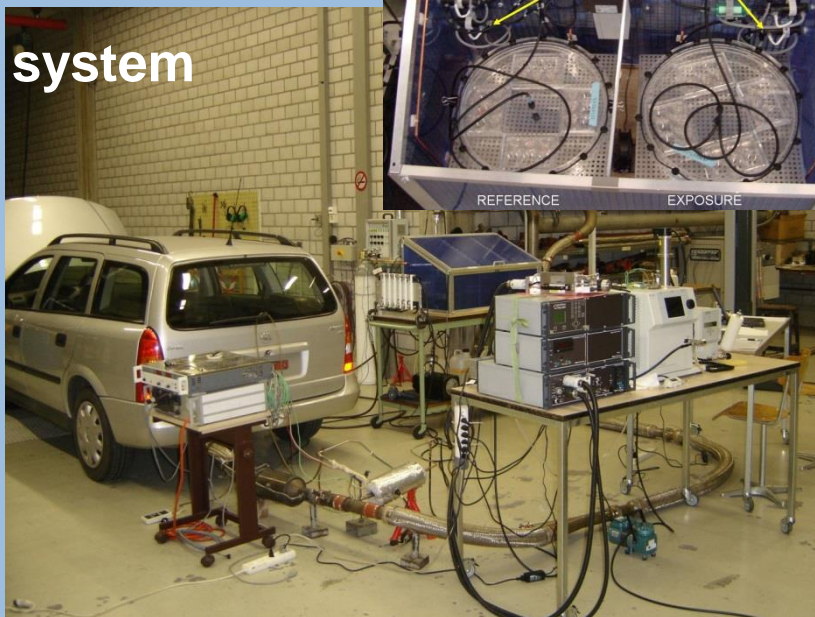


- (A: Phagocytosis)
- B: Macropinocytosis
- C: Clathrin-mediated endocytosis
- D: Clathrin and caveolae independent endocytic pathways
- E: Caveolae-mediated endocytosis**
- F: Adhesive interaction (entering):** *interaction of nanoparticles with cell membrane, effect on fluidity, nanoparticles may slip into cell between phospholipid molecules (→ U. Nienhaus, KIT)*

Brandenberger et al., Small, 2010

EFFECTS OF DIESEL EXHAUST ON BIOLOGICAL SYSTEMS

Exhaust system



Exposure system

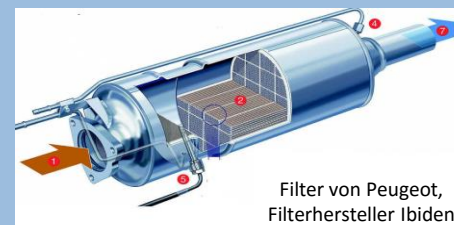
- Opel Astra X20DTL, 35 km/h
- Fuel: low sulfur diesel (>10mg/kg, Greenergy SA)
- Lube oil (V10.237, Motorex)
- Exhaust dilution 1:10

⇒ Without particle filter

⇒ With a silicon carbide diesel particle filter

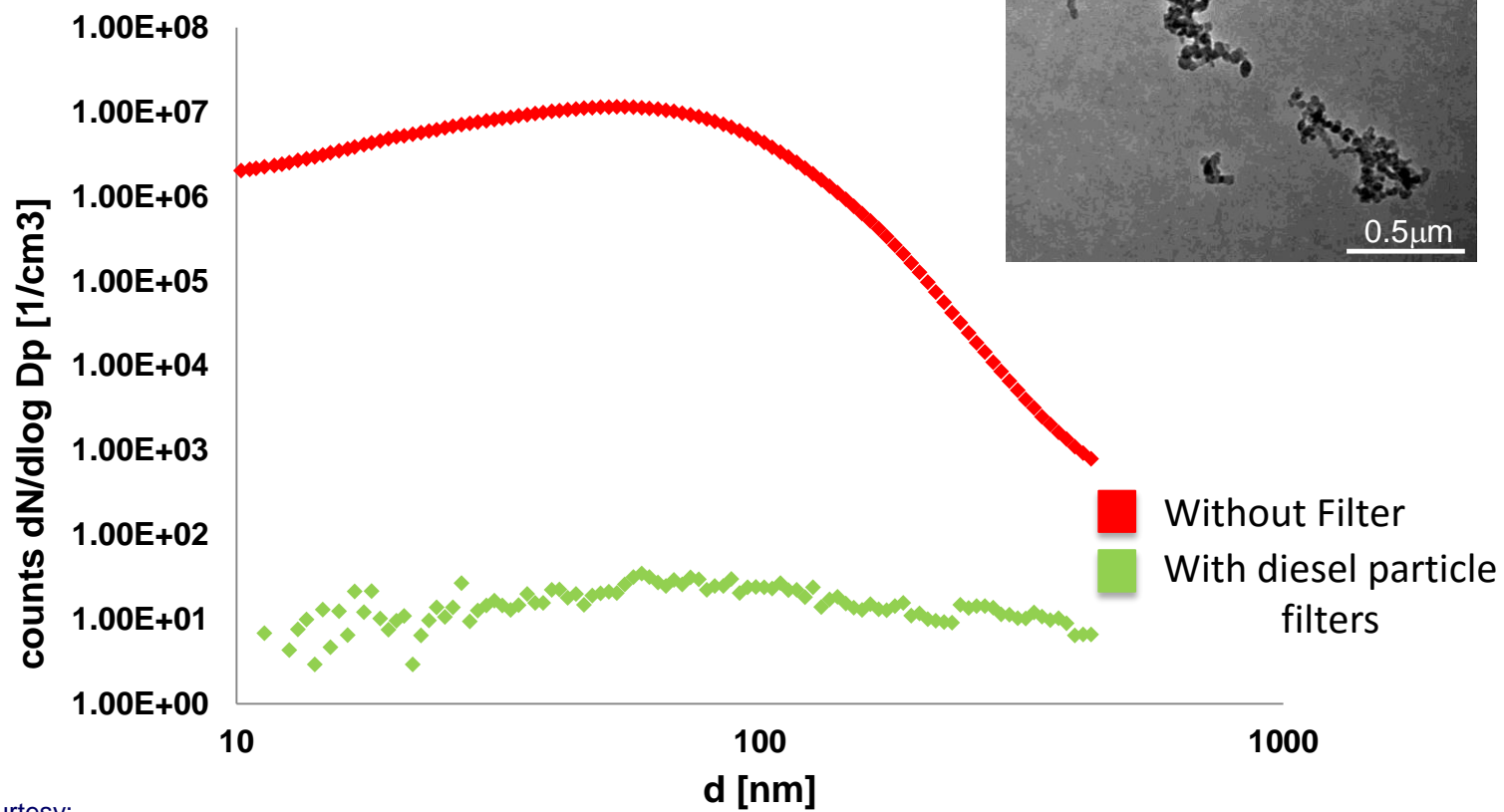
Müller et al. Environ Sci Technol 2009;
Steiner et al. Tox Letters 2012 in press

Courtesy:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland



DIESEL EXHAUST I

PARTICLE SIZE DISTRIBUTION

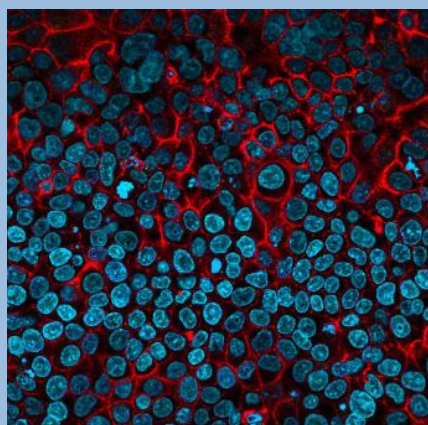


Courtesy:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland

Steiner et al., Atmos. Environ., 2013

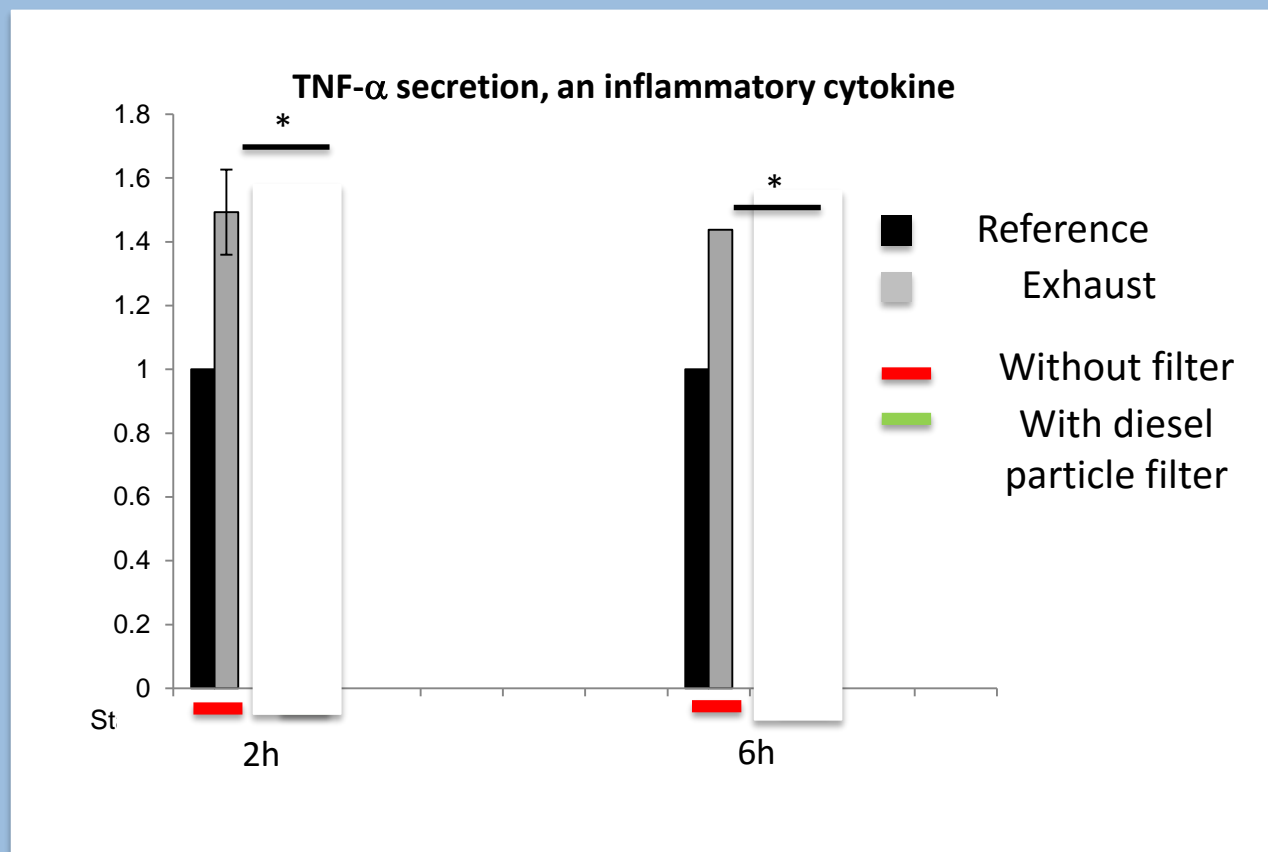
DIESEL EXHAUST II

INFLAMMATORY REACTION OF CELLS



Confocal light micrograph
(blue: nuclei, red: actin)

Courtesy:
Barbara Rothen-Rutishauser
Adolphe Merkle Institute
University of Fribourg
Switzerland



Steiner et al., Atmos. Environ., 2013

UFP SUMMARY

COMBUSTION AEROSOLS HAVE EFFECTS ON
LUNG FUNCTION/HEALTH (PARTICLE-LUNG INTERACTION)

u^b

b
UNIVERSITÄT
BERN

- Deposition on internal surfaces of the lungs
- Displacement of particles towards epithelial layer by surfactant at air-aqueous phase interface (surface forces)
- Interaction with pulmonary cells (epithelial, defence system): cellular interplay, intracellular trafficking
- Translocation through air-blood tissue barrier into capillary blood
- Tanslocation to secondary organs by blood circulation
- **Particle size matters!**

WHAT HAS TO BE CONSIDERED OF NANOPARTICLES FROM COMBUSTION GENERATED AEROSOLS

- **Size of particles** (nanoparticles)
- **Displacement of nanoparticles** towards epithelial layer (surfactant, surface forces)
- **Distance to capillaries** (translocation to secondary organs)
- **Distance to sensitive cells** (interaction), effect: e.g. immune modulation?
- **Interaction with cells** (uptake/penetration, effect: oxidative stress, inflammatory reaction, immune modulation, uncontrolled cell division, DNA damage a.o.?)